```
1     GCCGTCACTCCCTCCGTCATCGATAACATCCTGTCCAAGATCGAGAACGAGTACG
1▶    Ala Val Thr Pro Ser Val Ile Asp Asn Ile Leu Ser Lys Ile Glu Asn Glu Tyr G
56    AGGTGCTGTACCTGAAGCCGCTGGCAGGGGTCTACCGGAGCCTGAAGAAGCAG
19▶   lu Val Leu Tyr Leu Lys Pro Leu Ala Gly Val Tyr Arg Ser Leu Lys Lys Gln
109   CTGGAGAACAACGTGATGACCTTCAACGTGAACGTGAAGGATATCCTGAACAGC
37▶   Leu Glu Asn Asn Val Met Thr Phe Asn Val Asn Val Lys Asp Ile Leu Asn Ser
163   CGGTTCAACAAGCGGGAGAACTTCAAGAACGTGCTGGAGAGCGATCTGATCCC
55▶   Arg Phe Asn Lys Arg Glu Asn Phe Lys Asn Val Leu Glu Ser Asp Leu Ile Pr
216   CTACAAGGATCTGACCAGCAGCAACTACGTGGTCAAGGATCCCTACAAGTTCC
72▶   o Tyr Lys Asp Leu Thr Ser Ser Asn Tyr Val Val Lys Asp Pro Tyr Lys Phe L
269   TGAACAAGGAGAAGAGAGATAAGTTCCTGAGCAGTTACAACTACATCAAGGATAG
90▶   eu Asn Lys Glu Lys Arg Asp Lys Phe Leu Ser Ser Tyr Asn Tyr Ile Lys Asp Se
324   CATTGATACCGATATCAACTTCGCCAACGATGTCCTGGGATACTACAAGATCCT
108▶  r Ile Asp Thr Asp Ile Asn Phe Ala Asn Asp Val Leu Gly Tyr Tyr Lys Ile Le
378   GTCCGAGAAGTACAAGAGCGATCTGGATTCAATCAAGAAGTACATCAACGATAA
126▶  u Ser Glu Lys Tyr Lys Ser Asp Leu Asp Ser Ile Lys Lys Tyr Ile Asn Asp Ly
432   GCAGGGAGAGAACGAGAAGTACCTGCCCTTCCTGAACAACATCGAGACCCTGTA
144▶  s Gln Gly Glu Asn Glu Lys Tyr Leu Pro Phe Leu Asn Asn Ile Glu Thr Leu Ty
486   CAAGACCGTCAACGATAAGATTGATCTGTTCGTGATCCACCTGGAGGCCAAGGT
162▶  r Lys Thr Val Asn Asp Lys Ile Asp Leu Phe Val Ile His Leu Glu Ala Lys Va
                          NdeI
540   CCTGAACTACACATATGAGAAGAGCAACGTGGAGGTCAAGATCAAGGAGCTGAA
180▶  l Leu Asn Tyr Thr Tyr Glu Lys Ser Asn Val Glu Val Lys Ile Lys Glu Leu As
594   TTACCTGAAGACCATCCAGGATAAGCTGGCCGATTTCAAGAAGAACAACAACTT
198▶  n Tyr Leu Lys Thr Ile Gln Asp Lys Leu Ala Asp Phe Lys Lys Asn Asn Asn Ph
648   CGTCGGGATCGCCGATCTGAGCACCGATTACAACCACAACAACCTGCTGACCAA
216▶  e Val Gly Ile Ala Asp Leu Ser Thr Asp Tyr Asn His Asn Asn Leu Leu Thr Ly
702   GTTCCTGAGCACCGGTATGGTCTTCGAAAACCTGGCCAAGACCGTCCTGAGCAA
234▶  s Phe Leu Ser Thr Gly Met Val Phe Glu Asn Leu Ala Lys Thr Val Leu Ser As
756   CCTGCTGGATGGGAACCTGCAGGGGATGCTGAACATCAGCCAGCACCAGTGTGT
252▶  n Leu Leu Asp Gly Asn Leu Gln Gly Met Leu Asn Ile Ser Gln His Gln Cys Va
810   GAAGAAGCAGTGTCCCCAGAACAGCGGGTGTTTCAGACACCTGGATGAGAGAGA
270▶  l Lys Lys Gln Cys Pro Gln Asn Ser Gly Cys Phe Arg His Leu Asp Glu Arg Gl
864   GGAGTGTAAGTGTCTGCTGAACTACAAGCAGGAAGGTGATAAGTGTGTGGAAAAC
288▶  u Glu Cys Lys Cys Leu Leu Asn Tyr Lys Gln Glu Gly Asp Lys Cys Val Glu Asn
919   CCCAATCCTACTTGTAACGAGAACAATGGTGGATGTGATGCCGATGCCAAGTGTACCG
307▶  Pro Asn Pro Thr Cys Asn Glu Asn Asn Gly Gly Cys Asp Ala Asp Ala Lys Cys Thr G
977   AGGAGGATTCAGGGAGCAACGGGAAGAAGATCACCTGTGAGTGTACCAAGCCTGATT
326▶  lu Glu Asp Ser Gly Ser Asn Gly Lys Lys Ile Thr Cys Glu Cys Thr Lys Pro Asp S
1034  CTTATCCACTGTTCGATGGTATCTTCTGTAGT
345▶  er Tyr Pro Leu Phe Asp Gly Ile Phe Cys Ser
```

FIG. 1

```
   1 GCAGTAACTCCTTCCGTAATTGATAACATACTTTCTAAAATTGAAAATGAATA
   1▶AlaValThrProSerValIleAspAsnIleLeuSerLysIleGluAsnGluTyrG
                      EcoNI (73)
  56 AGGTTTTATATTTAAAACCTTTAGCAGGTGTTTATAGAAGTTTAAAAAAACAATT
  19▶luValLeuTyrLeuLysProLeuAlaGlyValTyrArgSerLeuLysLysGlnLe
 111 AGAAAATAACGTTATGACATTTAATGTTAATGTTAAGGATATTTTAAATTCACGA
  37▶uGluAsnAsnValMetThrPheAsnValAsnValLysAspIleLeuAsnSerArg
 166 TTTAATAAACGTGAAAATTTCAAAAATGTTTTAGAATCAGATTTAATTCCATATA
  56▶PheAsnLysArgGluAsnPheLysAsnValLeuGluSerAspLeuIleProTyrL
 221 AAGATTTAACATCAAGTAATTATGTTGTCAAAGATCCATATAAATTTCTTAATAA
  74▶ysAspLeuThrSerSerAsnTyrValValLysAspProTyrLysPheLeuAsnLy
 276 AGAAAAAAGAGATAAATTCTTAAGCAGTTATAATTATATTAAGGATTCAATAGAT
  92▶sGluLysArgAspLysPheLeuSerSerTyrAsnTyrIleLysAspSerIleAsp
 331 ACGGATATAAATTTTGCAAATGATGTTCTTGGATATTATAAAATATTATCCGAAA
 111▶ThrAspIleAsnPheAlaAsnAspValLeuGlyTyrTyrLysIleLeuSerGluL
 386 AATATAAATCAGATTTAGATTCAATTAAAAAATATATCAACGACAAACAAGGTGA
 129▶ysTyrLysSerAspLeuAspSerIleLysLysTyrIleAsnAspLysGlnGlyGl
 441 AAATGAGAAATACCTTCCCTTTTTAAACAATATTGAGACCTTATATAAAACAGTT
 147▶uAsnGluLysTyrLeuProPheLeuAsnAsnIleGluThrLeuTyrLysThrVal
 496 AATGATAAAATTGATTTATTTGTAATTCATTTAGAAGCAAAAGTTCTAAATTATA
 166▶AsnAspLysIleAspLeuPheValIleHisLeuGluAlaLysValLeuAsnTyrT
 551 CATATGAGAAATCAAACGTAGAAGTTAAAATAAAAGAACTTAATTACTTAAAAAC
 184▶hrTyrGluLysSerAsnValGluValLysIleLysGluLeuAsnTyrLeuLysTh
 606 AATTCAAGACAAATTGGCAGATTTTAAAAAAAATAACAATTTCGTTGGAATTGCT
 202▶rIleGlnAspLysLeuAlaAspPheLysLysAsnAsnAsnPheValGlyIleAla
 661 GATTTATCAACAGATTATAACCATAATAACTTATTGACAAAGTTCCTTAGTACAG
 221▶AspLeuSerThrAspTyrAsnHisAsnAsnLeuLeuThrLysPheLeuSerThrG
 716 GTATGGTTTTTGAAAATCTTGCTAAAACCGTTTTATCTAATTTACTTGATGGAAA
 239▶lyMetValPheGluAsnLeuAlaLysThrValLeuSerAsnLeuLeuAspGlyAs
 771 CTTGCAAGGTATGTTAAACATTTCACAACACCAATGCGTAAAAAAACAATGTCCA
 257▶nLeuGlnGlyMetLeuAsnIleSerGlnHisGlnCysValLysLysGlnCysPro
 826 CAAAATTCTGGATGTTTCAGACATTTAGATGAAAGAGAAGAATGTAAATGTTTAT
 276▶GlnAsnSerGlyCysPheArgHisLeuAspGluArgGluGluCysLysCysLeuL
 881 TAAATTACAAACAAGAAGGTGATAAATGTGTTGAAAATCCAAATCCTACTTGTAA
 294▶euAsnTyrLysGlnGluGlyAspLysCysValGluAsnProAsnProThrCysAs
 936 CGAAAATAATGGTGGATGTGATGCAGATGCCAAATGTACCGAAGAAGATTCAGGT
 312▶nGluAsnAsnGlyGlyCysAspAlaAspAlaLysCysThrGluGluAspSerGly
 991 AGCAACGGAAAGAAAATCACATGTGAATGTACTAAACCTGATTCTTATCCACTTT
 331▶SerAsnGlyLysLysIleThrCysGluCysThrLysProAspSerTyrProLeuP
                  PstI    (1059)
1046 TCGATGGTATTTTCTGCAGTCACCACCACCACCACCACTAACT
 349▶heAspGlyIlePheCysSerHisHisHisHisHisHis•••
```

FIG. 2

Codon	AA	goat b-casein	goat K-casein	MSP wt	Edited MSP	mouse b-casein	mouse a-casein	mouse g-casein	mouse e-casein
TTT	Phe	5	4	8	0	4	8	3	4
TTC	Phe	4	3	7	15	4	6	7	1
TTA	Leu	0	2	25	0	0	0	0	0
TTG	Leu	0	2	3	0	0	0	0	1
TCT	Ser	5	1	4	1	13	5	7	5
TCC	Ser	2	2	2	3	6	14	8	2
TCA	Ser	1	4	10	1	1	3	2	0
TCG	Ser	0	1	0	0	0	0	0	0
TAT	Tyr	2	7	17	2	1	3	2	1
TAC	Tyr	1	2	3	18	2	6	6	7
TAA	***	1	2	0	0	1	0	1	0
TAG	***	0	0	0	0	0	0	0	0
TGT	Cys	1	1	10	12	0	0	1	0
TGC	Cys	0	2	2	0	2	2	2	1
TGA	***	0	0	0	0	0	1	0	1
TGG	Trp	1	1	0	0	0	2	2	2
CTT	Leu	9	1	9	0	16	9	3	3
CTC	Leu	5	2	0	0	7	8	0	1
CTA	Leu	1	2	1	0	1	2	1	0
CTG	Leu	11	5	0	38	10	17	4	1
CCT	Pro	17	6	4	2	8	6	3	0
CCC	Pro	12	0	1	6	8	6	6	4
CCA	Pro	3	13	5	1	5	6	2	2
CCG	Pro	1	1	0	1	0	0	0	1
CAT	His	0	1	3	0	2	6	2	1
CAC	His	5	3	1	4	4	0	3	0
CAA	Gln	5	9	9	0	9	21	9	7
CAG	Gln	16	6	0	9	21	32	12	8
CGT	Arg	0	1	1	0	0	0	0	0
CGC	Arg	0	0	0	0	1	0	0	0
CGA	Arg	0	0	1	0	0	0	0	1
CGG	Arg	1	0	0	3	0	0	0	0
ATT	Ile	4	5	13	0	3	4	3	4
ATC	Ile	6	3	2	20	7	5	8	5
ATA	Ile	1	3	5	0	1	0	2	0
ATG	Met	7	3	3	3	4	12	2	13
ACT	Thr	7	6	3	2	6	5	1	4
ACC	Thr	2	7	3	13	4	4	4	4
ACA	Thr	2	4	9	1	1	1	2	0
ACG	Thr	0	0	1	0	0	0	2	0
AAT	Asn	2	6	29	3	4	6	3	1
AAC	Asn	2	3	12	38	4	9	4	6
AAA	Lys	7	6	38	0	6	7	3	5
AAG	Lys	6	4	4	42	3	6	13	7
AGT	Ser	2	6	5	2	3	6	6	5
AGC	Ser	5	0	2	16	2	6	6	3
AGA	Arg	2	2	4	3	1	8	1	1
AGG	Arg	0	2	0	0	0	0	0	1
GTT	Val	5	6	15	0	7	4	2	3
GTC	Val	8	2	1	11	7	3	3	0
GTA	Val	2	2	5	0	2	4	1	3
GTG	Val	8	4	0	10	6	3	5	3
GCT	Ala	1	3	2	0	8	17	4	2
GCC	Ala	4	7	1	8	6	3	3	3
GCA	Ala	3	7	6	1	4	13	1	1
GCG	Ala	0	1	0	0	0	0	0	0
GAT	Asp	4	5	25	27	3	6	4	2
GAC	Asp	0	2	2	0	1	2	1	3
GAA	Glu	10	6	21	3	6	12	9	6
GAG	Glu	9	5	4	22	5	5	5	5
GGT	Gly	2	1	8	4	0	0	0	0
GGC	Gly	0	0	0	0	0	0	0	0
GGA	Gly	2	1	6	3	1	0	1	0
GGG	Gly	1	0	0	7	1	0	0	0

FIG. 3A

Codon	AA	MSP wt	Edited MSP	MSP wt	Edited MSP	E.coli	Human
TTT	Phe	8	0	0.53	0	0.5	0.35
TTC	Phe	7	15	0.47	1	0.5	0.65
TTA	Leu	25	0	0.66	0	0.11	0.05
TTG	Leu	3	0	0.08	0	0.11	0.09
TCT	Ser	4	1	0.17	0.04	0.27	0.17
TCC	Ser	2	3	0.09	0.13	0.21	0.26
TCA	Ser	10	1	0.43	0.04	0.13	0.11
TCG	Ser	0	0	0	0	0.14	0.07
TAT	Tyr	17	2	0.85	0.1	0.54	0.47
TAC	Tyr	3	18	0.15	0.9	0.46	0.53
TAA	***	0	0				
TAG	***	0	0				
TGT	Cys	10	12	0.83	1	0.45	0.3
TGC	Cys	2	0	0.17	0	0.55	0.7
TGA	***	0	0				
TGG	Trp	0	0	0	0	1	1
CTT	Leu	9	0	0.24	0	0.12	0.11
CTC	Leu	0	0	0	0	0.12	0.22
CTA	Leu	1	0	0.02	0	0.03	0.07
CTG	Leu	0	38	0	1	0.72	0.46
CCT	Pro	4	2	0.4	0.2	0.14	0.24
CCC	Pro	1	6	0.1	0.6	0.11	0.41
CCA	Pro	5	1	0.5	0.1	0.2	0.24
CCG	Pro	0	1	0	0.1	0.54	0.11
CAT	His	3	0	0.75	0	0.64	0.42
CAC	His	1	4	0.25	1	0.36	0.58
CAA	Gln	9	0	1	0	0.31	0.26
CAG	Gln	0	9	0	1	0.69	0.74
CGT	Arg	1	0	0.17	0	0.46	0.09
CGC	Arg	0	0	0	0	0.32	0.19
CGA	Arg	1	0	0.17	0	0.05	0.1
CGG	Arg	0	3	0	0.5	0.06	0.15
ATT	Ile	13	0	0.65	0	0.39	0.23
ATC	Ile	2	20	0.1	1	0.52	0.64
ATA	Ile	5	0	0.25	0	0.08	0.13
ATG	Met	3	3	1	1	1	1
ACT	Thr	3	2	0.19	0.13	0.36	0.2
ACC	Thr	3	13	0.19	0.81	0.38	0.47
ACA	Thr	9	1	0.56	0.06	0.09	0.21
ACG	Thr	1	0	0.06	0	0.17	0.12
AAT	Asn	29	3	0.71	0.07	0.29	0.34
AAC	Asn	12	38	0.29	0.93	0.71	0.66
AAA	Lys	38	0	0.9	0	0.72	0.45
AAG	Lys	4	42	0.1	1	0.28	0.55
AGT	Ser	5	2	0.21	0.09	0.11	0.11
AGC	Ser	2	16	0.09	0.7	0.14	0.29
AGA	Arg	4	3	0.67	0.5	0.08	0.24
AGG	Arg	0	0	0	0	0.03	0.23
GTT	Val	15	0	0.71	0	0.37	0.13
GTC	Val	1	11	0.05	0.52	0.12	0.27
GTA	Val	5	0	0.24	0	0.28	0.09
GTG	Val	0	10	0	0.48	0.23	0.5
GCT	Ala	2	0	0.22	0	0.33	0.31
GCC	Ala	1	8	0.11	0.89	0.18	0.4
GCA	Ala	6	1	0.67	0.11	0.28	0.17
GCG	Ala	0	0	0	0	0.21	0.12
GAT	Asp	25	27	0.93	1	0.48	0.38
GAC	Asp	2	0	0.07	0	0.52	0.62
GAA	Glu	21	3	0.84	0.12	0.67	0.4
GAG	Glu	4	22	0.16	0.88	0.33	0.6
GGT	Gly	8	4	0.57	0.29	0.46	0.15
GGC	Gly	0	0	0	0	0.4	0.44
GGA	Gly	6	3	0.43	0.21	0.06	0.17
GGG	Gly	0	7	0	0.5	0.08	0.24

FIG. 3B

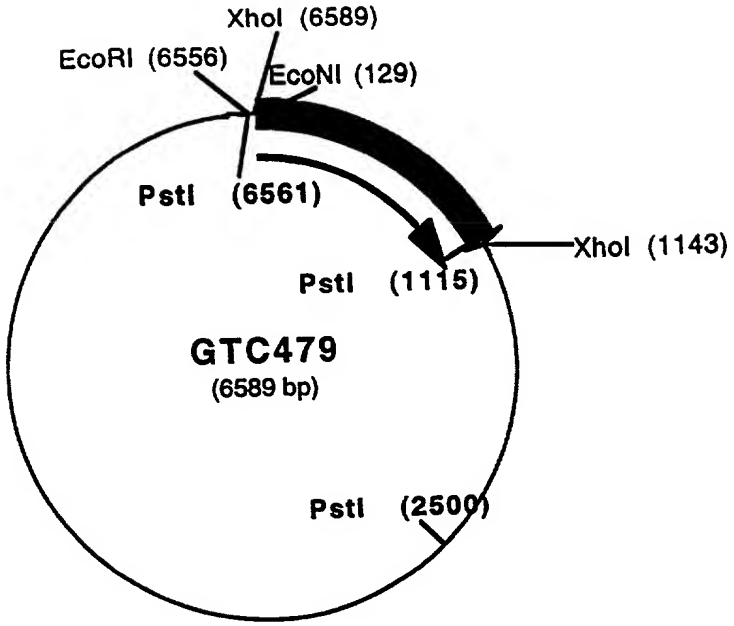
XhoI (6589)
EcoRI (6556)
EcoNI (129)
PstI (6561)
XhoI (1143)
PstI (1115)
GTC479
(6589 bp)
PstI (2500)

FIG. 4A

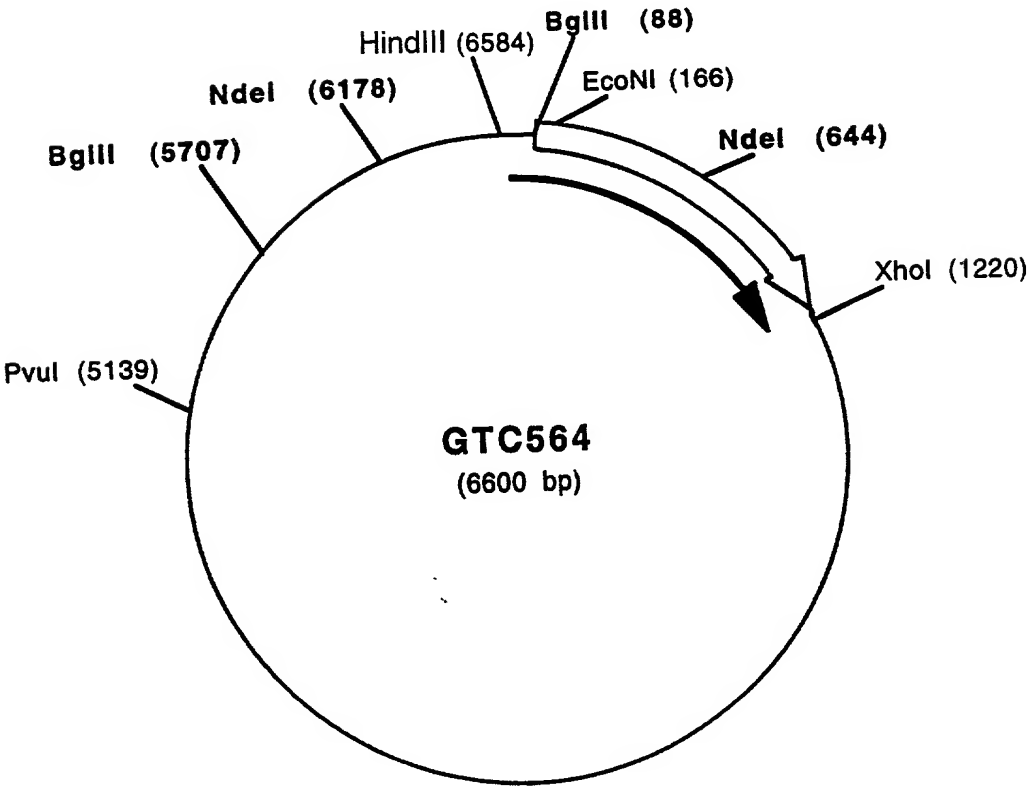
BglII (88)
HindIII (6584)
EcoNI (166)
NdeI (6178)
NdeI (644)
BglII (5707)
XhoI (1220)
PvuI (5139)
GTC564
(6600 bp)

FIG. 4B

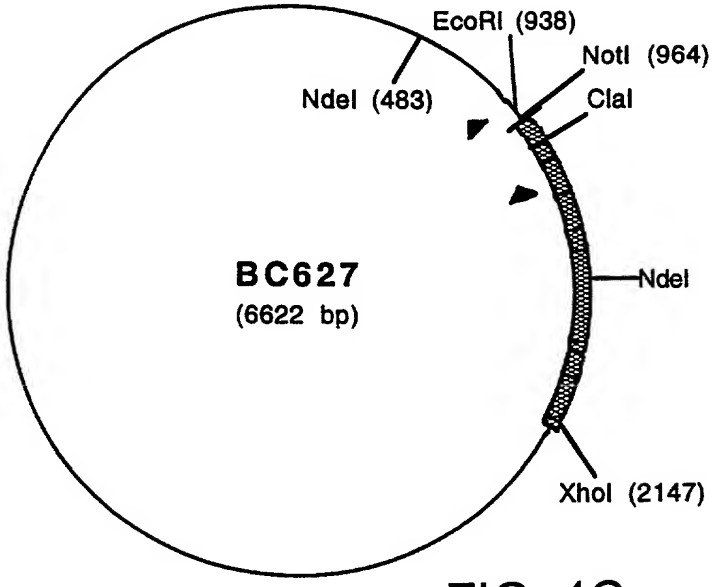

FIG. 4C

Oligos used:

OT1:
TCG ACG AGA GCC ATG AAG GTC CTC ATC CTT GCC TGT CTG GTG GCT CTG GCC ATT GCA AGA GAG CAG GAA GAA CTC AAT GTA GTC GGT A,

OT2:
GAT CTA CCG ACT ACA TTG AGT TCT TCC TGC TCT CTT GCA ATG GCC AGA GCC ACC AGA CAG GCA AGG ATG AGG ACC TTC ATG GCT CTC G,

MSP1:
AATAGATCTGCAGTAACTCCTTCCGTAATTG,

MSP2:
AATTCTCGAGTTAGTGGTGGTGGTGGTGGTGACTGCAGAAATACCATC

MSP8:
TAACTCGAGCGAACCATGAAGGTCCTCATCCTTGCCTGTCTGGTGGCTCTGG CCATTGCA

FIG. 6

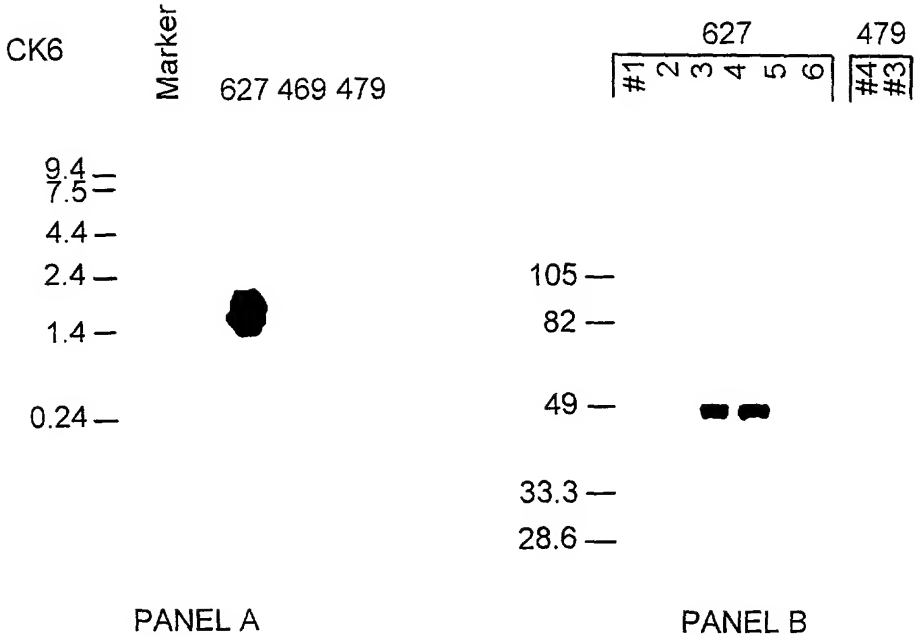

FIG. 5

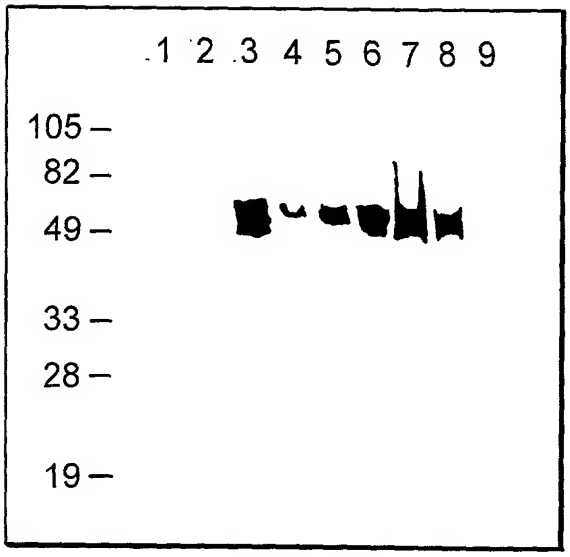

FIG. 10

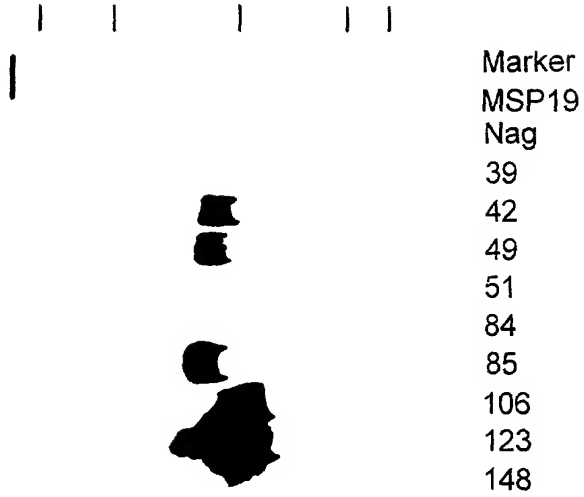

FIG. 13

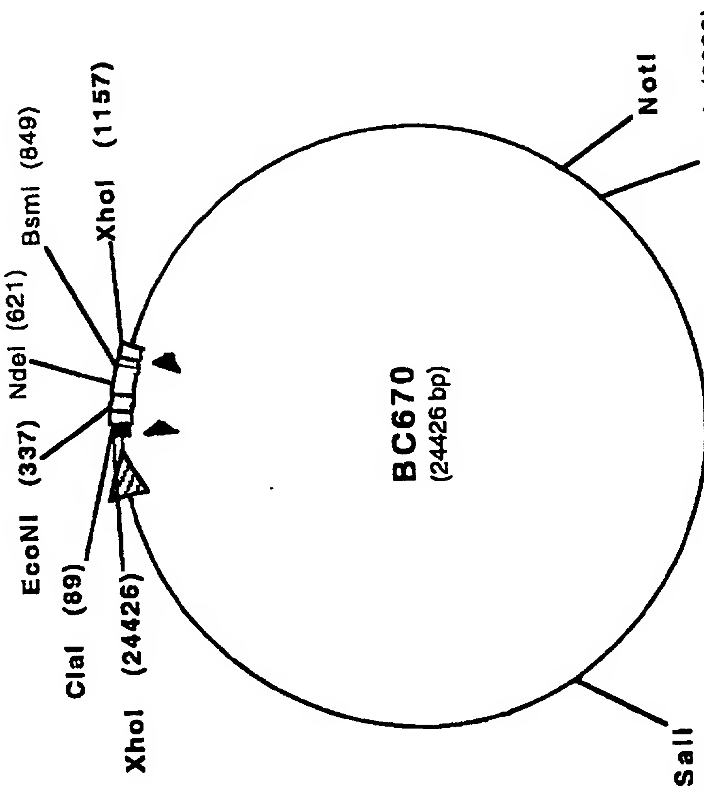

FIG. 8

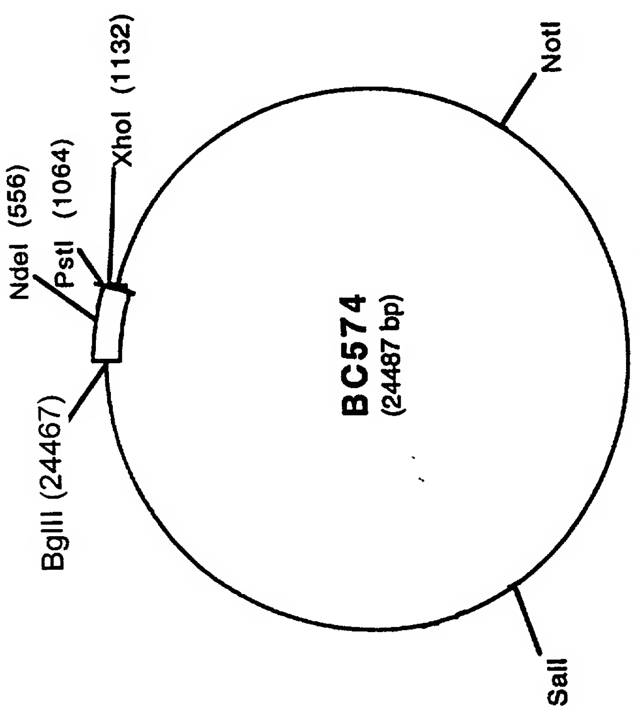

FIG. 7

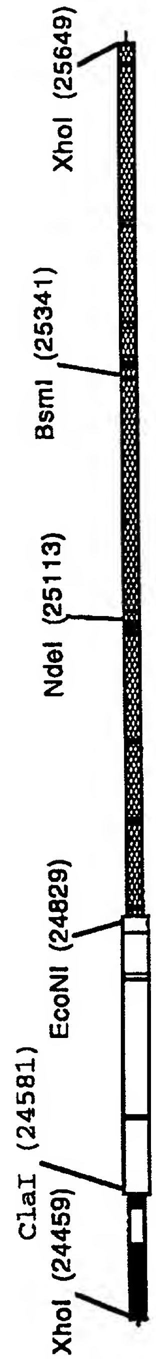

Diagram of BC620

FIG. 9

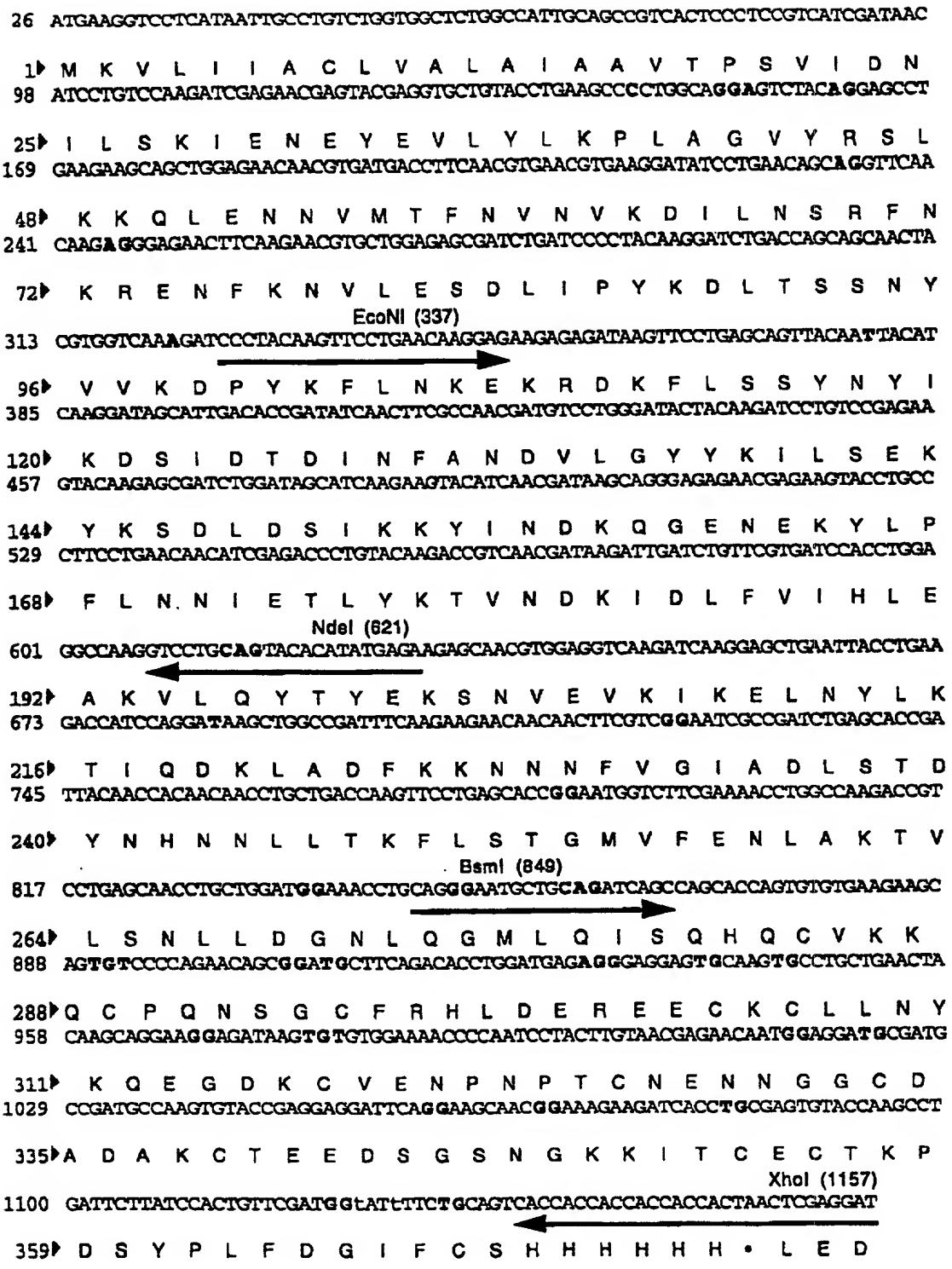
26 ATGAAGGTCCTCATAATTGCCTGTCTGGTGGCTCTGGCCATTGCAGCCGTCACTCCCTCCGTCATCGATAAC
1 M K V L I I A C L V A L A I A A V T P S V I D N
98 ATCCTGTCCAAGATCGAGAACGAGTACGAGGTGCTGTACCTGAAGCCCCTGGCAGGAGTCTACAGGAGCCT
25 I L S K I E N E Y E V L Y L K P L A G V Y R S L
169 GAAGAAGCAGCTGGAGAACAACGTGATGACCTTCAACGTGAACGTGAAGGATATCCTGAACAGCAGGTTCAA
48 K K Q L E N N V M T F N V N V K D I L N S R F N
241 CAAGAGGGAGAACTTCAAGAACGTGCTGGAGAGCGATCTGATCCCCTACAAGGATCTGACCAGCAGCAACTA
72 K R E N F K N V L E S D L I P Y K D L T S S N Y
EcoNI (337)
313 CGTGGTCAAAGATCCCTACAAGTTCCTGAACAAGGAGAAGAGAGATAAGTTCCTGAGCAGTTACAATTACAT
96 V V K D P Y K F L N K E K R D K F L S S Y N Y I
385 CAAGGATAGCATTGACACCGATATCAACTTCGCCAACGATGTCCTGGGATACTACAAGATCCTGTCCGAGAA
120 K D S I D T D I N F A N D V L G Y Y K I L S E K
457 GTACAAGAGCGATCTGGATAGCATCAAGAAGTACATCAACGATAAGCAGGGAGAGAACGAGAAGTACCTGCC
144 Y K S D L D S I K K Y I N D K Q G E N E K Y L P
529 CTTCCTGAACAACATCGAGACCCTGTACAAGACCGTCAACGATAAGATTGATCTGTTCGTGATCCACCTGGA
168 F L N N I E T L Y K T V N D K I D L F V I H L E
NdeI (621)
601 GGCCAAGGTCCTGCAGTACACATATGAGAAGAGCAACGTGGAGGTCAAGATCAAGGAGCTGAATTACCTGAA
192 A K V L Q Y T Y E K S N V E V K I K E L N Y L K
673 GACCATCCAGGATAAGCTGGCCGATTTCAAGAAGAACAACAACTTCGTCGGAATCGCCGATCTGAGCACCGA
216 T I Q D K L A D F K K N N N F V G I A D L S T D
745 TTACAACCACAACAACCTGCTGACCAAGTTCCTGAGCACCGGAATGGTCTTCGAAAACCTGGCCAAGACCGT
240 Y N H N N L L T K F L S T G M V F E N L A K T V
BsmI (849)
817 CCTGAGCAACCTGCTGGATGGAAACCTGCAGGGAATGCTGCAGATCAGCCAGCACCAGTGTGTGAAGAAGC
264 L S N L L D G N L Q G M L Q I S Q H Q C V K K
888 AGTGTCCCCAGAACAGCGGATGCTTCAGACACCTGGATGAGAGGGAGGAGTGCAAGTGCCTGCTGAACTA
288 Q C P Q N S G C F R H L D E R E E C K C L L N Y
958 CAAGCAGGAAGGAGATAAGTGTGTGGAAAACCCCAATCCTACTTGTAACGAGAACAATGGAGGATGCGATG
311 K Q E G D K C V E N P N P T C N E N N G G C D
1029 CCGATGCCAAGTGTACCGAGGAGGATTCAGGAAGCAACGGAAAGAAGATCACCTGCGAGTGTACCAAGCCT
335 A D A K C T E E D S G S N G K K I T C E C T K P
XhoI (1157)
1100 GATTCTTATCCACTGTTCGATGGTATTTCTGCAGTCACCACCACCACCACCACTAACTCGAGGAT
359 D S Y P L F D G I F C S H H H H H H • L E D

FIG. 11

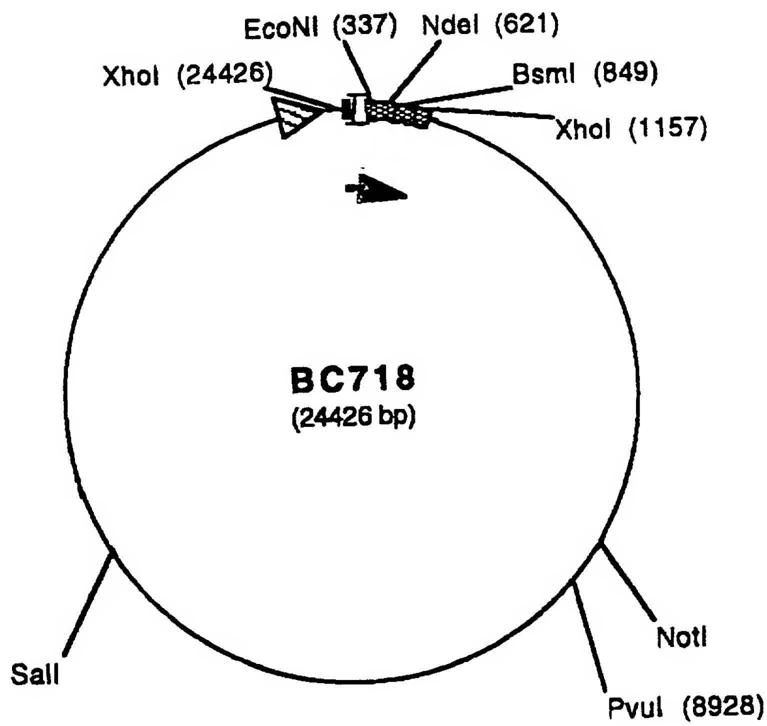
EcoNI (337)
NdeI (621)
XhoI (24426)
BsmI (849)
XhoI (1157)
BC718
(24426 bp)
SalI
NotI
PvuI (8928)

FIG. 12